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Exclusion of the Stomatin, α-Adducin and β-Adducin Loci in a Large Kindred With Dehydrated Hereditary Stomatocytosis

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Defects in stomatin, α-adducin, and β-adducin have been implicated in erythrocyte disorders of cation permeability. We performed linkage analysis of the genetic loci for these proteins in a large kindred with xerocytosis (dehydrated hereditary stomatocytosis). Using polymerase chain reaction-based genotyping techniques, all three loci are excluded as disease gene candidates. Am. J. Hematol. 60:72–74, 1999. © 1999 Wiley-Liss, Inc.

Key words: stomatocytosis; erythrocytes; linkage analysis; stomatin; alpha-adducin; beta-adducin

INTRODUCTION

Hereditary stomatocytosis (HSt) is a heterogeneous group of disorders characterized by mouth-shaped (stomatocytic) erythrocyte morphology on peripheral blood smear. The clinical severity of HSt is variable; some patients experience hemolysis and anemia, whereas others are asymptomatic [1]. The red blood cell membranes of HSt patients usually exhibit abnormal permeability to the univalent cations sodium and potassium, with resultant modification of intracellular water content.

Variants of HSt have been described [1]. In the overhydrated (hydrocytosis) variant, a net gain of sodium and potassium cause water to enter, forming swollen, overhydrated erythrocytes. In the dehydrated (xerocytosis) variant, loss of monovalent cations produces shrunken, dehydrated cells. Other hereditary hemolytic anemias associated with abnormal cation permeability and red cell hydration span a continuum between overhydrated and dehydrated. None are precisely defined in clinical, molecular, or genetic terms.

Stomatin, a 31 kDa integral membrane phosphoprotein also known as band 7.2b, is absent from the erythrocyte membranes of patients with overhydrated HSt [2] and is decreased in some HSt variants, including dehydrated HSt [3]. It is unknown whether stomatin's absence is the cause of the erythrocyte abnormalities in HSt, or whether it is simply a consequence of the underlying genetic abnormality. Although the function of stomatin is not completely understood, it most likely acts as a *trans* regulator of a cation channel, because increased rates of diffusion occur in its absence [4]. Recent studies also demonstrate a potential interaction between stomatin and adducin, a component of the membrane skeleton [5]. It is hypothesized that stomatin participates in a variety of specialized cellular functions including mechanosensory signal transduction, and that its action is linked to the cell's cytoskeleton by the α,β adducin heterodimer [6]. The stomatin gene is located at 9q34.1; α-adducin at 4p16.3; and β-adducin at 2p13-p14.

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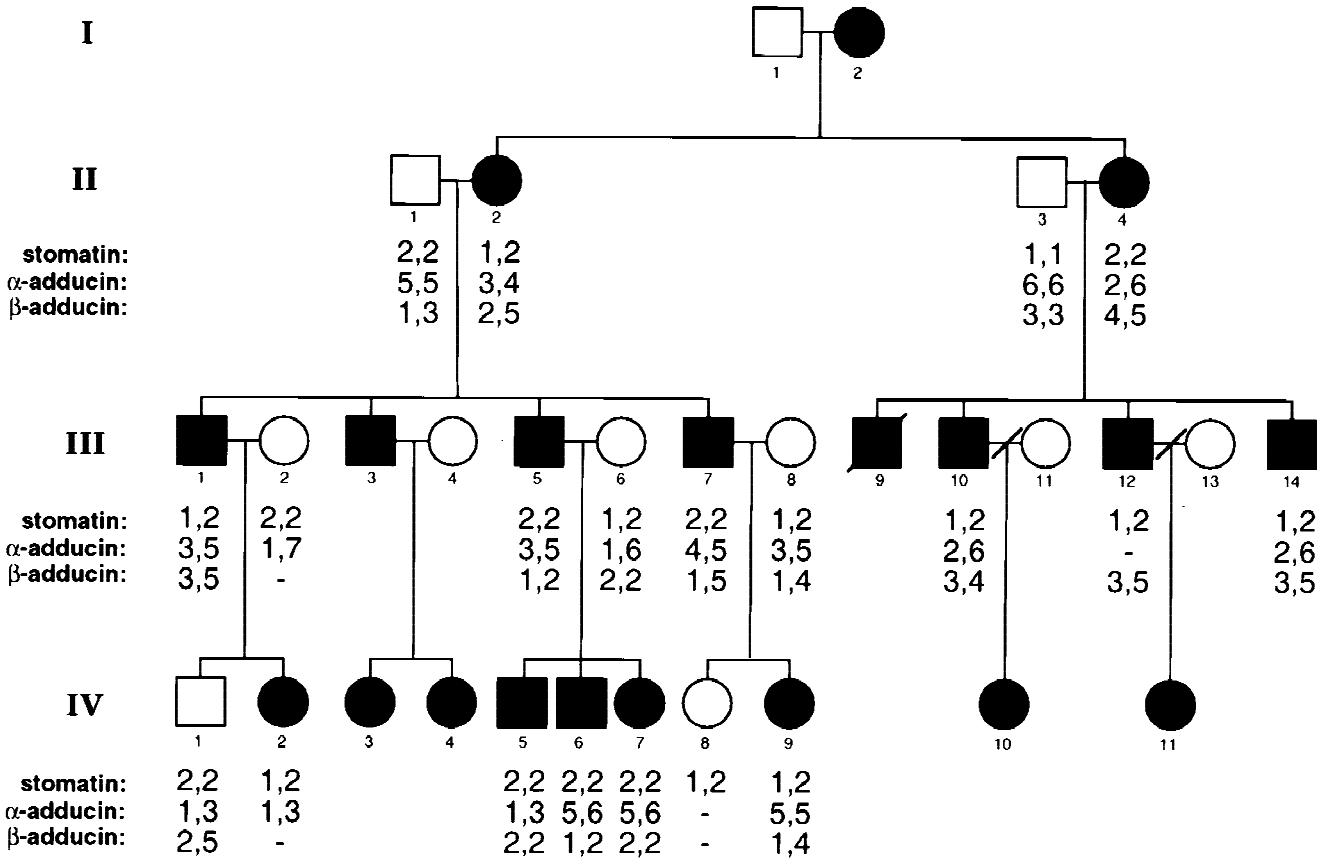

Fig. 1. Pedigree of the dehydrated hereditary stomatocytosis kindred. Filled symbols indicate affected individuals. A "–" indicates that the alleles did not amplify. For stomatin, affected II-2 passes the "1" allele to affected III-1 but the "2" allele to affected III-5 and III-7. The disease cosegregates with the "2" allele in the right half of the pedigree, and it is a "2" allele that would have to carry the disease from III-7 to IV-9. However, affected IV-2 inherits a "1" allele from affected III-1, indicating a lack of linkage between the disease and the stomatin marker. Alpha-adducin is also excluded at multiple points. Affected II-2 and II-4 do not share a common allele. II-2 passes allele "3" to III-1 and III-5 but allele "4" to III-7, yet all offspring are affected. Affected III-1 passes the same allele to both children (allele "3"), yet only one of the two offspring are affected. Beta-adducin is also excluded at multiple points in the pedigree. Affected II-2 and II-4 do not share a common allele. III-5 and III-7 inherit different alleles from affected II-2, yet both offspring are affected. Similarly, III-10 and III-12 inherit different alleles from affected II-4, yet both offspring are affected.

To investigate the potential role of these proteins in the pathogenesis of dehydrated HSt, we performed genotyping of the stomatin, α-adducin and β-adducin loci on genomic DNA samples from members of a large kindred with dehydrated HSt.

METHODS AND RESULTS

The family studied is a well-characterized, dehydrated HSt kindred with members spanning three generations. The HSt phenotype was assigned to family members as previously described [7]. Blood was collected with informed consent.

Genomic DNA was isolated from peripheral blood lymphocytes using standard techniques. Polymerase chain reaction (PCR) amplification was performed using 100 ng of genomic DNA, 0.5 μg of each primer, 2 nmoles dNTP mix, 0.1 μl [^{32}P]-dCTP, 1 μl 10× PCR buffer, and 0.1 μl *Taq* polymerase in a 10 μl reaction volume. Twenty-six cycles of amplification were performed with annealing at 58°C for 30 sec. The oligonucleotide primers used to genotype the stomatin locus, corresponding to an intron within the stomatin gene, were: 5′-CCTTTTATGACTTTTGCTCC-3′ and 5′-AGGTCTTAATTGTGGGGAGG-3′ [8]. The primers used to investigate α-adducin, corresponding to the D4S95 locus just 3′ to the α-adducin gene, were: 5′-GCATAAAA TGGGGATAACAGTAC-3′ and 5′-GACATTGCTTTATAGCTGTGCCTCAGTTT-3′ [9,10]. The primers used to amplify the β-adducin locus, corresponding to D2S291 (GDB Accession ID: 199210) in the 5′ flanking region of the β-adducin gene, were: 5′-TGGCCCCAAGTTGGATTT-3′ and 5′-CCCCTAGCCATCCTAGACG-3′. Amplification products were dena-

tured, electrophoresed in a 5% polyacrylamide gel, and subjected to autoradiography.

Amplified alleles were arbitrarily assigned numbers and the genotype of each family member at each locus was assessed (Fig. 1). All results were internally consistent in that each allele could be traced to one of the parents. No alleles from any of the three loci cosegregated with the disease phenotype. Based on these data, all three genes can be excluded as candidate disease genes for this disease.

DISCUSSION

Stomatin is absent in the erythrocytes of patients with overhydrated HSt, and variably deficient in patients with other HSt variants [3,11]. However, the role of stomatin in the transmission of HSt has not been adequately assessed because of insufficient pedigree size [1]. The present pedigree overcomes this limitation, and allowed by linkage analysis an assessment of the contribution of the stomatin gene to the transmission of HSt. In this manner, stomatin was excluded as the gene responsible for HSt.

In a similar manner, because of recent data suggesting a functional linkage between stomatin and the cytoskeletal protein adducin, genetic markers tightly linked to the α-adducin and β-adducin loci were used to genotype members of the pedigree. These genes also showed no relation to the inheritance of HSt.

Because of the great clinical, biochemical, and genetic heterogeneity in the HSt syndromes, it will be important to perform linkage to include or exclude these three loci in other HSt kindreds. In this kindred, with these three candidate genes excluded, attention can turn to other loci implicated in the transmission of HSt.

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